Centre For AIDS Reagents

Data Sheet

NAME: pNL4-3 deltaENV-EGFP

REPOSITORY REFERENCE: **100616**

DESCRIPTION OF CLONE: This is a HIV-1 reporter construct. It is derived from the HIV-1 proviral clone pNL4-3 but carries a form of enhanced green fluorescent protein (EGFP) in the *env* open reading frame rendering Env non-functional.

This vector expresses an endoplasmic reticulum (ER)-retained truncated Env-EGFP fusion protein. The EGFP was amplified from the pEGFP-N1 plasmid (Clontech) with primers that introduced the endonuclease sites, a KDEL ER-retention signal and a stop codon at the 3' end. The PCR product was inserted in frame into the pNL4-3 backbone between the KpnI and NheI sites in the env gene.

ApaI and AgeI double digestion should produce one 1.5 Kb band and one 13.2 Kb band. MfeI digestion should produce one 3.9 Kb band, one 1.6 Kb band and one 9.2 Kb band. Desired *gag-pol* sequences can be inserted into this vector by ApaI-AgeI double digestion. Sequence and map

CHARACTERISTICS: This construct can be used for measuring viral replication in a pseudotyped single-round infection assays using GFP as the output reading. It can also be used to quantitatively measure HIV-1 drug resistance by replacing the *gag-pol*region of the construct with a desired sequence.

This reagent is currently being provided as purified DNA stabilized in DNAstable *PLUS* and dried. Please see the notice for additional information and the protocol for reconstitution of dried DNA reagents.

PRESENTATION: 5 µg of purified DNA stabilized in DNAstable *PLUS* and dried.

SOURCE: Drs. Haili Zhang, Yan Zhou, and Robert Siliciano. (courtesy of the NIH AIDS Research and Reference Reagent Program)

REFERENCE: Zhang H. et al. *J of Virology* **78**:1718-1729, 2004.

NOTE: **Patent Pending.**

Scientists at for-profit institutions or who intend commercial use of this reagent must contact Dr. Robert Siliciano at rsiliciano@jhmi.edu, (tel) 410-955-2958, (fax) 410-955-0964 and specify the name of the reagent and a description of the intended use of the reagent, before the reagent can be released.

ACKNOWLEDGEMENTS: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pNL4-3-deltaE-EGFP (Cat# 11100) from Drs. Haili Zhang, Yan Zhou, and Robert Siliciano." Also include the reference cited above in any publications.